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Molecular Conformation, Receptor Binding, and Hormone Action of Natural and Synthetic Estrogens and Antiestrogens

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The X-ray crystallographic structural determinations of synthetic estrogens and antiestrogens provide reliable information on the global minimum energy conformation of these molecules or a local minimum energy conformation that is within 1 or 2 kcal/mole of the global minimum. In favorable cases, state-of-the-art molecular mechanics calculations provide quantitative agreement with X-ray results and information on the relative energy of other local minimum energy conformations not observed crystallographically.

Because the conformation of diethylstilbestrol (DES) observed in solvated crystals has an overall conformation and dipole moment more similar to estradiol it is the form more likely to bind to the receptor and produce hormone activity. Either phenol ring of DES can successfully mimic the estradiol A-ring in binding to the receptor. Indenestrol A (INDA) and indenestrol B (INDB) have nearly identical fully extended planar conformations. Either the α or γ rings of these compounds may mimic the A ring of estradiol and compete for the estrogen receptor. Although there are eight distinct ways in which molecules of a racemic mixture of INDA or INDB can bind to the receptor, not all of them may be able to elicit a hormonal response. This may account for the reduced biological activity of the compounds despite their successful competition for receptor binding.

The minimum energy conformations of Z-pseudodiethylstilbestrol (ZPD) and E-pseudodiethylstilbestrol (EPD) are bent in a fashion similar to that of indanestrol (INDC). These molecules have good binding affinity suggesting that the receptor does not require a flat molecule. Therefore these conformations would appear to be compatible with receptor binding, but only the Z isomer has an energetically allowed extended conformation that accounts for its observed biological activity relative to DES.

Introduction

Steroid hormones are vital to numerous physiological processes including cell growth, sexual development, maintenance of salt balance, and sugar metabolism. Many of these activities are known to be contingent upon the binding of steroids to specific cytosolic protein receptors and the subsequent interaction of the steroid receptor complex with chromatin. Abnormalities in steroid hormone synthesis, metabolism and receptor interaction contribute to a variety of diseases. Functional analogs of steroids have been synthesized for therapeutic use in restoring or controlling endogenous hormone levels. Synthetic steroids are used extensively in fertility control, and antihormones that interfere with the synthesis, metabolism, and receptor binding of steroids are useful in some forms of chemotherapy. In addition, many other chemicals, drugs, and naturally occurring compounds have been shown to compete for the active sites of steroid-metabolizing enzymes or the binding site of steroid hormone receptors.

Because of the key role that steroids play in human health and disease therapy, a full understanding of the molecular details of steroid hormone action is essential. A careful examination of the molecular structures and three-dimensional shapes of the hormones, antihormones, chemicals, and drugs that compete for a common binding site on a specific receptor can provide information on binding and the structural requirements for the structural features that influence the extent and nature of the hormonal response.

X-ray crystallographic determinations provide a highly accurate picture of molecular geometry in a specific solid state environment. Studying the same compound in different crystal forms provides additional information on

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the molecular flexibility of a compound (*1*,*2*). For most uncharged organic molecules such as steroids, a structure observed in the solid state is at or very near a local minimum energy conformation. If the energy of the global minimum is 2 to 3 kcal/mole lower than that of any metastable state, it is highly probable that a crystal incorporating the minimum energy conformation will be formed preferentially. If two or more conformations of a flexible molecule are of nearly equal energy they will often form crystals incorporating both conformers either as crystallographically independent molecules with dissimilar environments (*3*) or as partial occupancy disordered molecules in a single site (*4*). Because the active forms of drugs and hormones may not necessarily be low energy forms it is important to have reliable information on the conformation and relative energies of molecules that may not be readily crystallizable. Such information can be derived from solution and gas phase measurements and theoretical calculations. X-ray crystallographic studies provide a useful guide to the interpretation of data from these other sources (*5*).

On the basis of a previous analysis of the structures of a variety of compounds that compete for binding to the estrogen receptor, we have proposed that the phenolic A ring of steroidal estrogens is primarily responsible for initiating receptor binding and that the D ring end of the steroid molecule is primarily responsible for governing biological activity (*6*). The following observations support this model. The only common feature of compounds that compete with estradiol for binding to the estrogen receptor with relatively high affinity is a phenolic ring. Simple compounds such as tetrahydronaphthol and p-*sec*-amyl phenol prevent or compete for the binding of estradiol to its receptor (*7*). Chernyaev et al. (*8*) have demonstrated that the removal of the 3-hydroxyl substituent significantly decreases receptor binding while retaining some portion of biological activity. Removal of the 17-hydroxyl was shown to decrease binding to a lesser extent than 3-hydroxyl removal but to almost totally abolish biological activity (*8*).

Anaylsis of the crystallographic data and molecular mechanics calculations for a series of estrogenic and antiestrogenic analogs, derivatives, and metabolites of diethylstilbestrol and triphenylethylene provide additional information concerning the structural basis for receptor binding and hormonal action of estrogens.

Estradiol and Diethylstilbestrol

On the basis of an examination of models of estradiol and the potent synthetic estrogen diethystilbestrol (DES) (Fig. 1*a* and *b*), Keasling and Schueler (*9*) proposed that the structural requirements for estrogenicity were a specific distance (14.5 Å) between two hydroxyl groups separated by a flat hydrophobic region. Subsequent X-ray crystallographic studies have revealed that the distances between the terminal oxygen groups in estradiol (*10*) and diethylstilbestrol (*11*) are 10.9 and 12.1 Å (Fig. 2*a* and *b*), respectively, and that the molecules (particularly DES) are not as flat as their conventional chemical drawings might suggest. The small but significant difference between the oxygen–oxygen distances in these structures cannot be overcome by

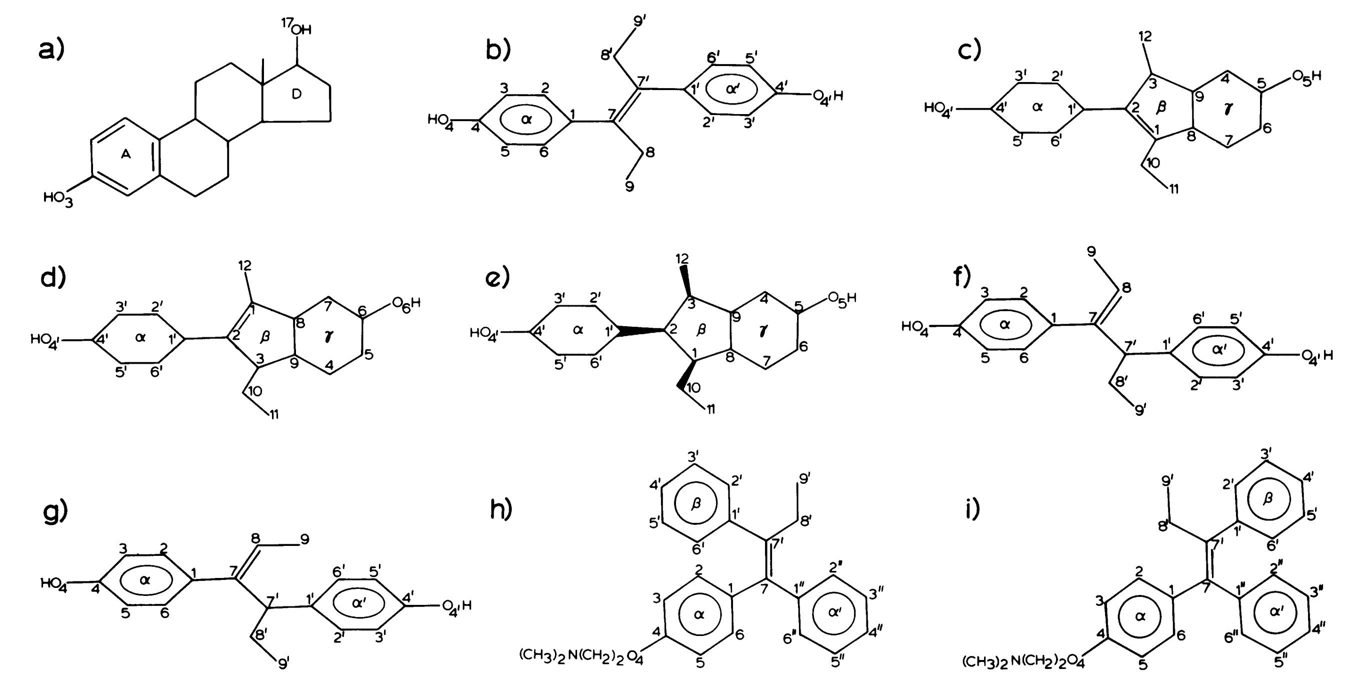

FIGURE 1. Chemical diagrams, atomic numbering and ring identification for (*a*) estradiol, (*b*) diethylstilbestrol, DES, (*c*) indenestrol A, INDA, (*d*) indenestrol B, INDB, (*e*) indanestrol, INDC, (*f*) Z-pseudo diethylstilbestrol (ZPD), (*g*) E-pseudo diethylstilbestrol (EPD), (*h*) *trans*- and (*i*) *cis*-tamoxifen.

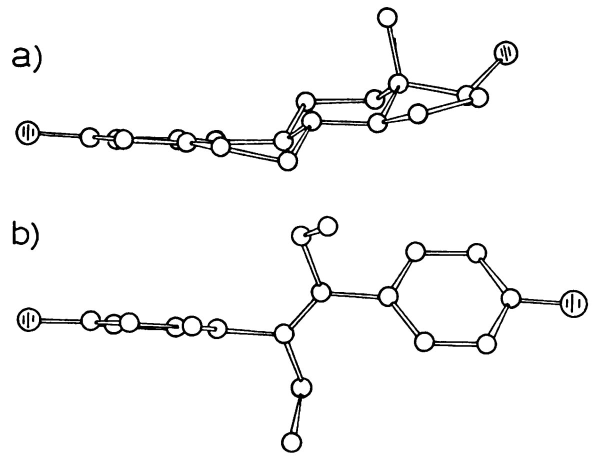

FIGURE 2. Comparison of the overall conformation of (*a*) estradiol, and (*b*) DES, viewed parallel to the plane of the A-ring in estradiol and one of the phenyl rings in DES.

molecular flexibility. The fairly rigid fused ring system of estradiol and the central double bond in DES prevent the oxygen from getting more than 0.1 Å closer to each other. If a specific distance between two hydroxyl groups is essential for estrogenic activity the 1.2 Å disparity between this distance in estradiol and DES could indicate that a water molecule plays a significant role in acting as a link between estradiol and the receptor (*6*). The distance between O(3) and a hydrogen-bonded oxygen in estradiol hydrate is 12.1 Å identical to the distance in DES (Fig. 3).

Although the oxygen-oxygen distance in DES is nearly fixed, conformational variation in the overall shape of the molecule is possible. Five distinct crystallographic observations of DES have resulted from studies of an anhydrous (*12*) and three solvated crystal forms (*11*). The DES molecule observed in the anhydrous crystal has crystallographically imposed inversion symmetry

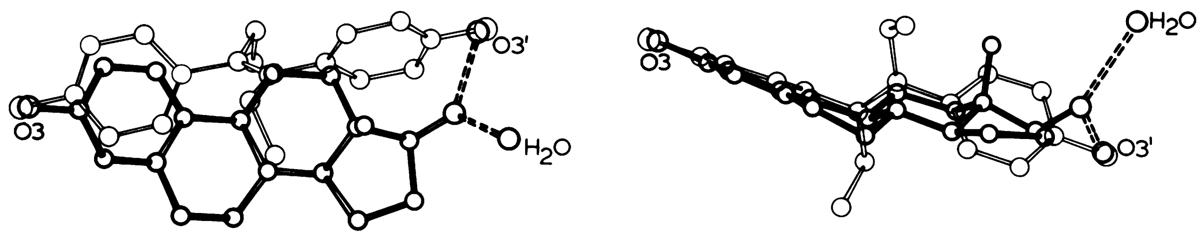

FIGURE 3. Superposition drawing of solvated estradiol and DES, maximizing relative positioning of hydrophilic groups and hydrophobic bulk. O3′ is the hydroxyl group of an adjacent molecule in crystals of estradiol. Hydrogen bonds are indicated as broken bonds.

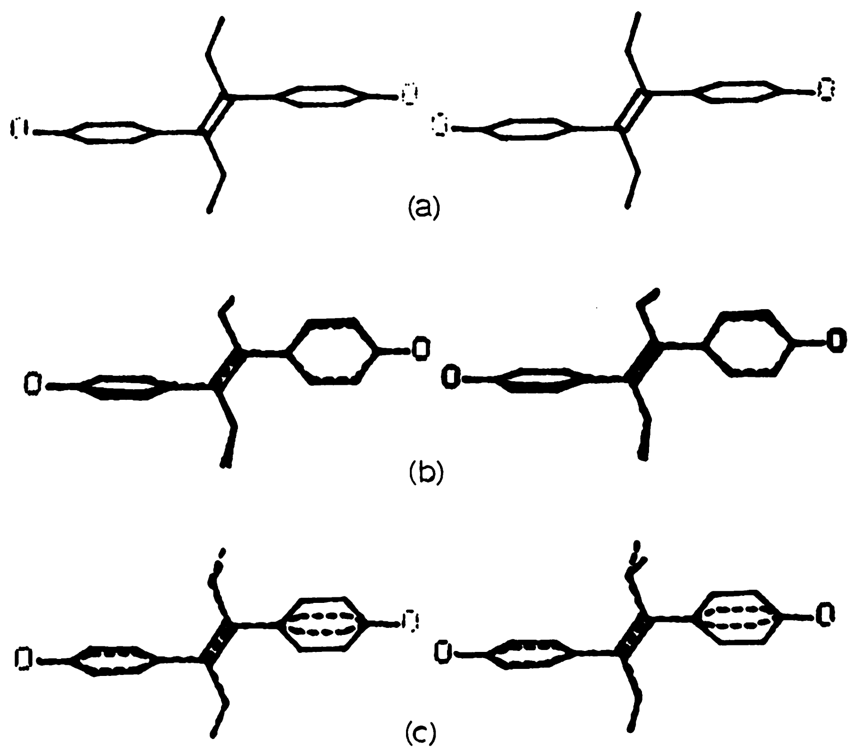

FIGURE 4. Stereo views of the crystallographically observed conformation of DES: (*a*) the conformation observed in anhydrous crystals, (*b*) a comparison of the conformation observed in three different solvated forms of DES and (*c*) the comparison of the conformation in the solvated (solid) and anhydrous (dashed) forms.

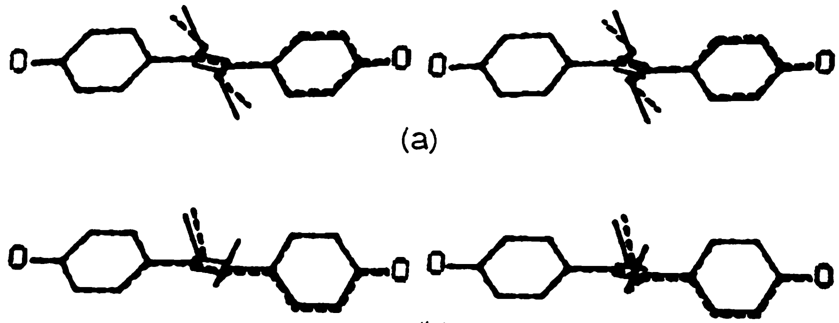

FIGURE 5. Stereo diagrams comparing the crystallographically observed conformations (solid) of (*a*) anhydrous and (*b*) solvated DES with the conformations predicted by molecular mechanics calculations to be local minima (dashed).

(S_2) about the midpoint of the ethylene bond (Fig. 4*a*). The molecules in the solvated crystal forms are significantly different from the anhydrous conformer, and nearly identical to one another (Fig. 4*b*). The solvated molecules have approximate C_2 symmetry with the rotation axis perpendicular to the plane of the ethylene bond and both of the methyl groups on the same side of the plane of the double bond. When the molecule from the anhydrous crystal is compared with one of the molecules from the solvated crystals (Fig. 4*c*), half of the molecule is seen to be nearly identical and the other half differs by correlated variations of the ϕ_2 and ϕ_3 torsion angles that results in a conversion from S_2 symmetry to C_2 symmetry. Neither of these conformations is particularly flat with the dihedral angles between the phenyl rings being 0° in the solvated molecule and 60° to 70° in the anhydrous form. The molecular mechanics program MM2p* was used to refine the conformational isomers of DES afforded by the X-ray determinations of solvated anhydrous crystal forms. The calculations are for the isolated molecules and no solvent present.

In Figure 5*a* and *b* the calculated molecules having C_{2h} and C_2 symmetry are compared with the corresponding crystallographically observed conformations by least-squares fitting of the atoms of one of the rings. This fit illustrates excellent overall agreement between the X-ray results and the calculations.The relative energies calculated for the anhydrous and the solvated molecules are 3.1 kcal/mole and 3.4 kcal/mole, and the calculated dipole moments are 0.0 and 2.2, respectively. The dipole moment of the molecule may well have a significant influence on complementarity of hormone-receptor interaction. The calculated dipole for estradiol in its solid state conformation is 2.3. The solid-state observation and the energy calculations of the two forms argues in favor of their being of comparable (near minimum) energy. Molecular environment will determine which of the two forms predominates. It is unlikely that the two conformations compete equally for the receptor site or are equally effective at eliciting hormonal response. The receptor may select one of the conformers. Hospital et al. (*15*) have concluded on the basis of hydrogen-bonding patterns and overall conformational features that it is the conformation observed in the solvated crystals that is responsible for both receptor binding and hormone action. The similar values of the dipole moments of estradiol and the DES conformer seen in the solvated crystals also supports the contention that it is this DES conformer that it most suited to mimicking estradiol in its binding to the estrogen receptor.

Table 1. Summary of reported binding affinities and uterotropic activities of estradiol (E), DES, indenestrol A(INDA), indenestrol B(INDB), and indanestrol (INDC).

Compound	C_{50}, nmole/L[a]	Doubling dose, μg/kg[b]
E	1.0 ± 0.1	10
DES	1.0 ± 0.1	7
INDA	0.7 ± 0.1	107
INDB	0.7 ± 0.2	111
INDC	50 ± 5	1120

[a]Nanomolar concentration of competitor required for 50% inhibition of specific binding of [^{3}H] estradiol. Results are expressed as the mean ± SE for a minimum of four determinations.

[b]Values are expressed as dose (μg/kg) of compound required to produce a 2-fold increase above the control of uterine weight/body weight ratio in 21-day-old CD-1 mice treated for 3 days. Data taken from Duax et al. (*20*).

Indanestrol and Indenestrol

A series of indanyl DES derivatives have been identified as *in vivo* metabolites of DES (*16*). These compounds possess effective receptor binding affinity but poor biological activity. Structures of the compounds indenestrol A (INDA) [1-ethyl-2(4′-hydroxyphenyl)-3-methyl-5-hydroxyindene] indenestrol B (INDB) [1-methyl-2(4′-hydroxyphenyl)-3-ethyl-6-hydroxyindene], and indanestrol (INDC) [1-ethyl-2-(4′-hydroxyphenyl)-3-methyl-5-hydroxyindane], are illustrated in Figure 1 (*c*, *d*, and *e*). The binding affinities of these compounds relative to those of estradiol (E) and DES as determined by competitive equilibrium receptor binding analysis and *in vivo* bioassay are presented in Table 1 (*17,18*).

There are three crystallographically independent molecules present in crystals of INDA (*18*). Although the molecules have slightly different crystalline environments and distinctly different hydrogen-bonding geometries, their conformations are nearly identical. The conformation of the three molecules of INDA are compared in Figure 6*a*. The atoms of the β and γ rings

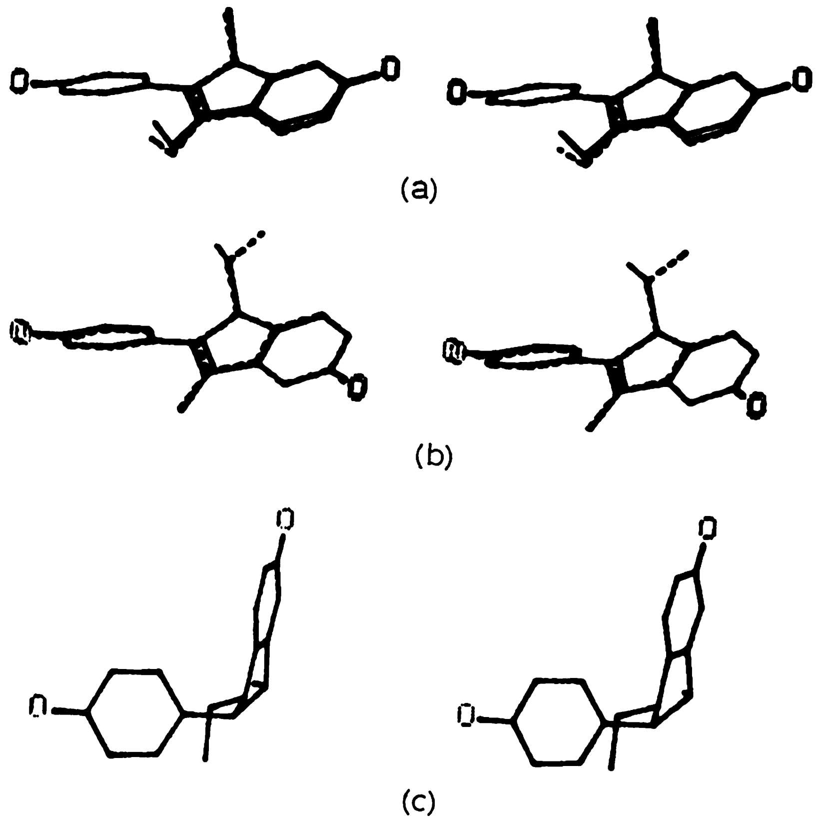

FIGURE 6. Stereo diagrams comparing the crystallographically observed conformation of (*a*) three conformers of INDA, (*b*) two conformers of INDB and (*c*) the single conformer of INDC. All structure determinations contain racemic pairs. Only one of the enantiomers in each pair is depicted above.

are coplanar and the α ring is twisted 27°, 31° and 33° out of that plane in the three crystallographically independent molecules. The C(3) methyl substituent and the terminal methyl of the ethyl substituent are on the same side of the plane of the β ring.

There are two crystallographically independent molecules present in crystals of INDB (*19*). The relative orientation of the planar rings are nearly identical to that observed in INDA. The β and γ rings are coplanar and the α rings are rotated 33° out of that plane. The two molecules of INDB differ significantly in the orientation of the C(11) methyl groups as illustrated in Figure 6*b*. The C-methyl atom is *trans* to the C(2)-C(3) bond in one molecule and oriented over the β ring, gauche to the C(2)-C(3) and C(3)-C(9) bond in the other.

The observed conformation of INDC is illustrated in Figure 6*c*. The methyl, ethyl, and phenyl substituents are all on the same side of the five-membered ring with the phenyl in an axial position and the ethyl and methyl substituents in equatorial positions. Because of the axial orientation of the phenyl substituent the molecule has an L-shape uncommon for the DES analogs.

The principle source of potential flexibility in the structures of INDA and INDB are rotations about the C(2)-C(1′) bonds and the orientation of the C(11) methyl group. The X-ray crystal structure results indicate that there is a highly restricted global minimum energy conformation in which the α ring is rotated away from coplanarity in order to strike a balance between unfavorable 1-4 and 1-5 nonbonding interactions and the energetic advantage of additional conjugation possible in a coplanar arrangement. The $30 \pm 2°$ rotation is in the direction that avoids unfavorable contacts between the α ring and the methyl substituent in INDA or the ethyl substituent on the INDB.

When the crystallographically observed conformations of INDA, INDB and INDC are subjected to potential energy minimization calculations using the MM2p program, the molecules undergo small changes at the two principal points of flexibility; rotations about the C(2)-C(1′) and the C(1)-C(10) or C(3)-C(10) bonds. The torsion angles involving these bonds in the calculated conformations differ by 2–17° from those observed crystallographically. The conformational isomers of INDB are calculated to have relative energies of 2.1 kcal/mole and 1.5 kcal/mole, in qualitative agreement with the nearly equal energy of the conformers suggested by their co-crystallization. The calculated and observed structures of INDA are compared in Figure 7, illustrating the excellent agreement in overall conformation.

When the conformation of the β ring in INDC was altered placing the α ring in an equatorial conformation, energy minimization suggested that this is a metastable form of 4.1 kcal/mole higher energy than the crystallographically observed conformation. This calculation is compatible with the lower binding affinity and hormonal activity of the INDC structure relative to INDA and INDB, but probably underestimates the energy difference.

Because INDA, INDB and INDC all contain two phenolic rings capable of mimicking the A ring of estradiol in its interaction with the receptor, each could conceivably bind in four different orientations. Because they are all racemic mixtures and there are four orientations possible for each enantiomer, there are eight ways that each could bind. The presence of the double bond in the five-membered ring of INDA and INDB forces them to have relatively flat, fully extended conformations in which both phenolic rings are exposed permitting ready access to either surface of either ring. For this reason they are found to compete for the estrogen receptor with affinities comparable to that of DES (*17,18*). The reduction in binding of INDC is almost certainly due to its bent ring L-shaped conformation. Although the phenolic rings could be expected to have some affinity for the binding site, the L-shape appears to interfere with optimal receptor interaction.

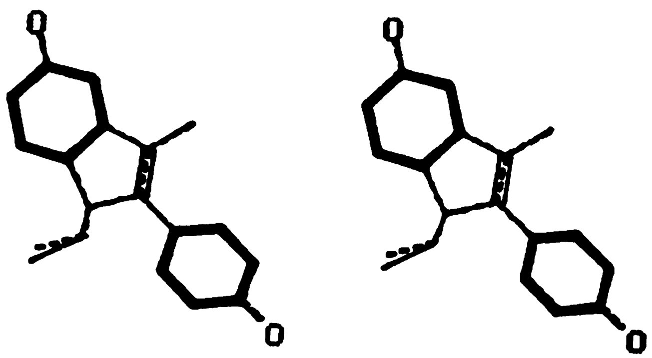

FIGURE 7. A comparison between the observed (solid) and calculated (dashed) structures of INDB.

Despite the fact that INDA and INDB compete successfully with DES for binding to the estrogen receptor they are found to have approximately 1/15 the uterotropic biological activity of DES. This significant reduction in activity could be due to the fact that (1) both enantiomers are not equivalent in biological activity and (2) not all of the four possible ligand binding orientations (Fig. 8) in the receptor are capable of inducing the biological activity of the receptor complex. If the γ ring of INDA or INDB were to mimic the A ring of estradiol (Fig. 8*c* and *d*) and bind to the receptor the hydroxyl on the α ring would be so far displaced from the 0(17) position that it would fail to fulfill the role of 0(17) in promoting hormone action. If only one of the eight binding modes possible for a racemic mixture of INDA (or INDB) has the proper orientation of the second hydroxyl group it would account for the reduction in biological activity that is observed despite evidence that INDA, INDB, and DES compete equally well *in vitro* for the estrogen steroid hormone binding site on the receptor.

Pseudo-Diethylstilbestrol

A diethyestrilbestrol analog, called pseudo-DES, in which the double bond is between C(7) and C(8) rather than C(7) and C(7′) can exist in Z and E configurations (Fig 1*f* and *g*). Biological testing revealed that while

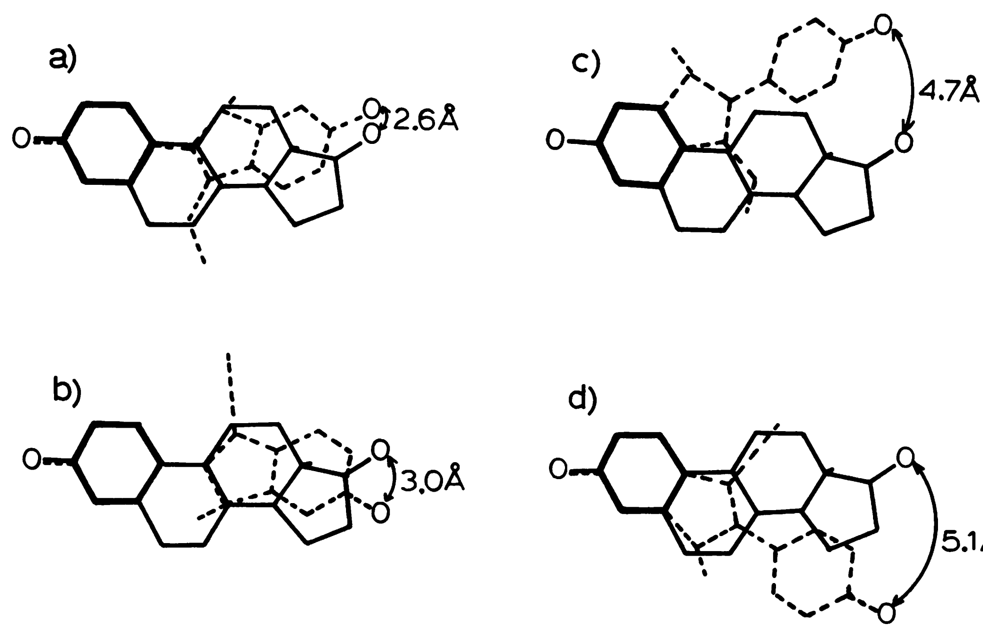

FIGURE 8. Illustration of the four principal orientations in which the α or γ rings of INDA and INDB can mimic the A ring of estradiol when binding to the estrogen receptor. The example illustrated is INDA (dashed) and estradiol (solid).

Table 2. Comparison of the torsion angles defining the crystallographically observed and energy minimized conformation of ZPD, EPD, and 1-OH-ZPD.

		Torsion angle range[a]			
		EPD	ZPD	1-OH-ZPD	Range
Observed in X-ray crystal structure determinations	ϕ_1	−48.4	−62.8	−55.8	14.4°
	ϕ_2	77.0	75.6	71.6	5.4°
	ϕ_3	54.5	49.6	51.5	4.9°
	ϕ_4	−58.4	−57.6	−57.7	0.8°
Calcd for structures refined using the MM2p program	ϕ_1	−45.3	−58.3	−59.1	13.8
	ϕ_2	79.6	77.3	74.0	5.6
	ϕ_3	56.7	64.5	64.4	7.8
	ϕ_4	−58.6	−62.5	−62.5	1.3

[a] ϕ_1 = C(2)-C(1)-C(7)-C(8), ϕ_2 = C(1)-C(7)-C(7′)-C(1′), ϕ_3 = C(7)-C(7′)-C(1′)C(6′), ϕ_4 = C(7)-C(7′)-C(8′)-C(9′); atomic numbering as in Figure 1*f* and 1*g*.

Table 3. Comparison between observed and calculated average values for the four torsion angles defining the conformation of ZPD, EPD, and 20-OH-ZPD.

	Observed		Calculated		$\Delta(\mathrm{calc}_{Avg}-\mathrm{obs}_{Avg})$
ϕ_1	−55.6	14.4	-59.1	13.8	3.5
ϕ_2	74.7	5.4	74.0	5.6	0.7
ϕ_3	51.9	4.9	64.4	7.8	12.3
ϕ_4	−57.9	0.8	-62.5	1.3	4.6

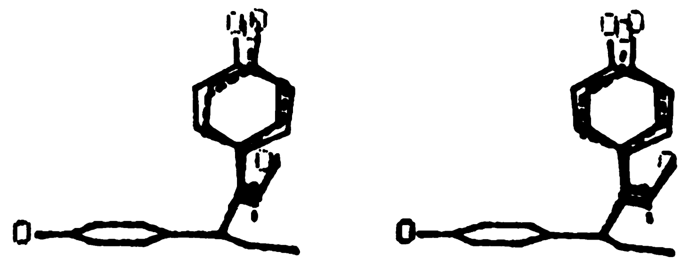

FIGURE 9. Stereo view of the superposition of ZPD, EPD, and 1-OH-ZPD, illustrating the similarity in their crystallographically observed conformations.

both isomers retained appreciable but different affinity for the estrogen receptor, the Z isomer (ZPD) has twice the uterotropic activity of the E (EPD) isomer (*21*). Since a significant conformational difference between the two might account for the observed activity difference, the X-ray crystal structure analyses of the two forms and the 1-OH derivative of ZPD were undertaken. The results revealed striking similarities in the observed conformation despite differences in composition, connectivity, hydrogen bonding and crystal packing. Four torsion angles fully define the overall conformation of these molecules (Table 2). The range in variation of three of these four torsion angles is 5° or less. The only significant variation is in the C(2)-C(1)-C(7)-C(8) torsion angle which differs by 14.4° between EPD and ZPD. The difference is a direct consequence of the sterochemical differences between the E and Z configurations. All three structures are observed to have a bent conformation in which the phenyl rings are oriented at right angles to one another (Fig. 9). This conformation is completely unlike the conformations of estradiol, DES and the indenestrol isomers, but bears some resemblance to indanestrol, a compound with little affinity for the receptor.

When the crystallographically observed conformations of EPD, ZPD and 1-OH-ZPD were subjected to energy minimization the structures retained their overall conformation with minor changes in their individual torsion angles (Tables 2 and 3). The standard deviation in the three observed ϕ_4 angles is only 0.8°, providing persuasive evidence that the conformation of this part of the molecule is highly inflexible, unperturbed by the Z to E variation, the addition of a hydroxyl group and the associated reorganization and crystal packing and all intermolecular contacts.

The consistency of the crystallographic results suggests that the bent form is the energy minimum conformation for ZPD, EPD and 1-OH ZPD. However, previous studies indicate that this conformation is unlikely to be compatible with any significant degree of estrogenic biological activity. For this reason it became important to determine whether an extended DES-like conformation might constitute a metastable state capable of inducing estrogenic biological response. The crystallographically observed structures of ZPD and EPD were transformed to the DES-like extended conformation and subjected to energy minimization. As a result of extremely close contacts between the hydrogens on C(9) and the α ring in EPD (Fig. 10*a*), the extended conformation is stereochemically untenable and the molecule refined to a bent conformation resembling in overall shape the crystallographically observed structure. In contrast to this, the extended conformation of the more active Z isomer does not incorporate intolerable nonbonding interactions and refinement indicates that a metastable state exists which resembles the DES conformation (Fig. 10*b*).

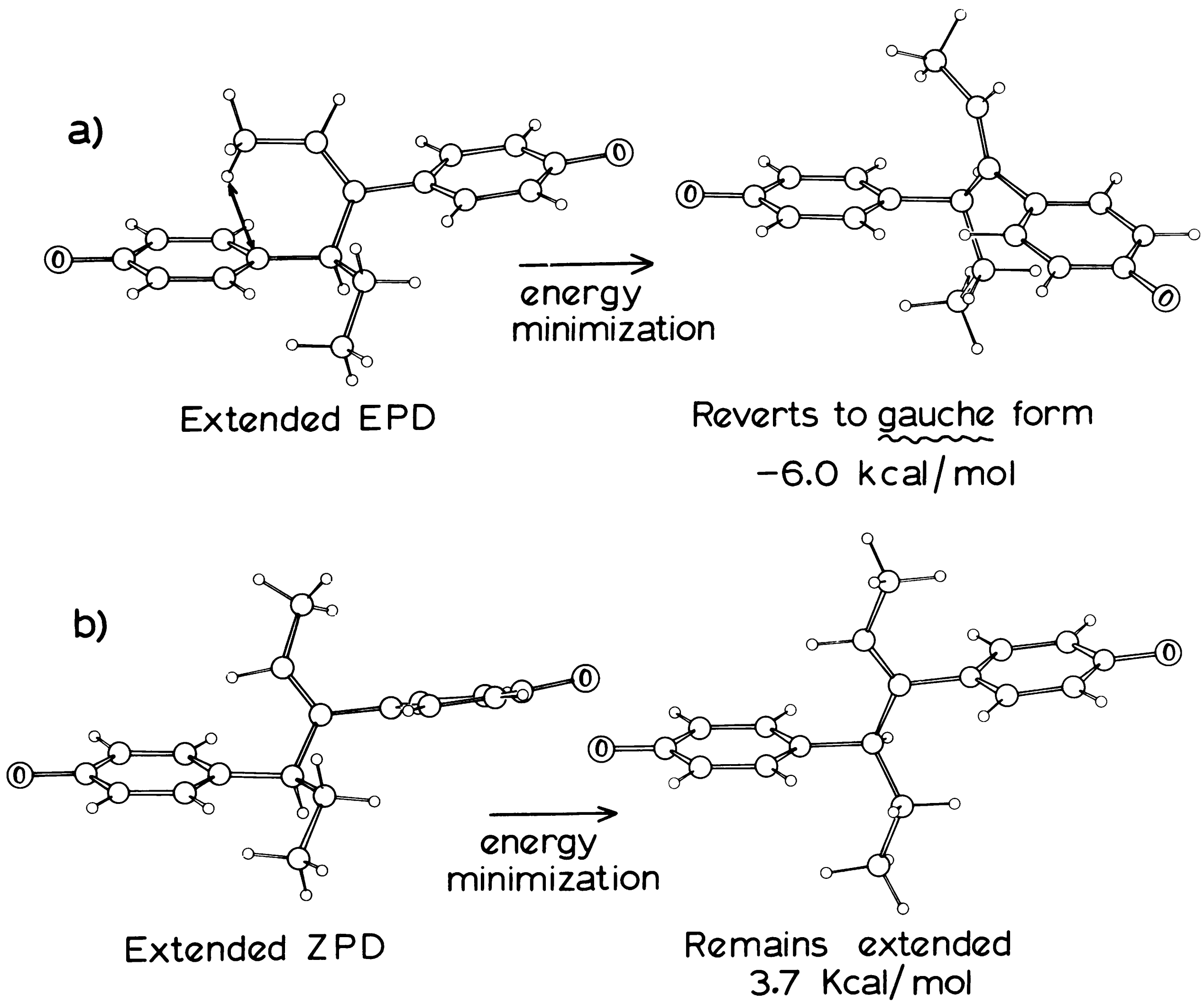

FIGURE 10. When theoretical DES-like extended conformers of (*a*) EPD and (*b*) ZPD were used as the starting point for energy minimization, EPD reverted to a bent conformation but ZPD retained an extended conformation.

Tamoxifen

A number of triphenylethylene derivatives exhibiting antiestrogenic properties are in use in cancer chemotherapy. The *trans* or Z isomer of tamoxifen (Fig. 1*h*) is one of the most potent antiestrogens. In contrast, the E isomer of tamoxifen (Fig. 1*i*) is reported to exhibit only weak estrogenic properties. The therapeutic properties of *trans* tamoxifen are primarily a result of the competition for binding to estrogen receptor of its major metabolite, 4-monohydroxy tamoxifen, where the hydroxyl has added to the 4″ position on the a′ ring (Fig. 1*h*). Although the X-ray crystal structure of 4-monohydroxy *trans* tamoxifen has not been reported, those of the *trans* (*22*) and *cis* (*3*) isomers of the parent compound provide a useful guide to the probable conformation of the active metabolite.

The E isomer (*cis*) crystallized with two conformationally distinct molecules in the crystal lattice (*3*). The two conformations (compared in Fig. 11) differ subtly in relative ring orientation and significantly in the conformation of the alkylamino ethoxy side chain. The relative ring orientations in the two isomers are almost identical as illustrated by the superposition of the β rings in the two structures shown in Figure 12.

The overall conformation of tamoxifen analogs can be defined by seven torsion angles, four relating the substituents on the ethylene group and three in the alkylaminoethoxy side chain. When the crystallographically observed conformations of Z and E isomers of tamoxifen are subjected to energy minimization, these torsion angles change by 5 to 25°. The most significant changes occur in the torsion angles defining the orientation of the phenyl rings relative to the ethylene group. The observed and refined conformations of the *cis* isomer are compared in Figure 13.

It is reasonable to suppose that relative to the estradiol A ring the phenol ring of monohydroxy tamoxifen

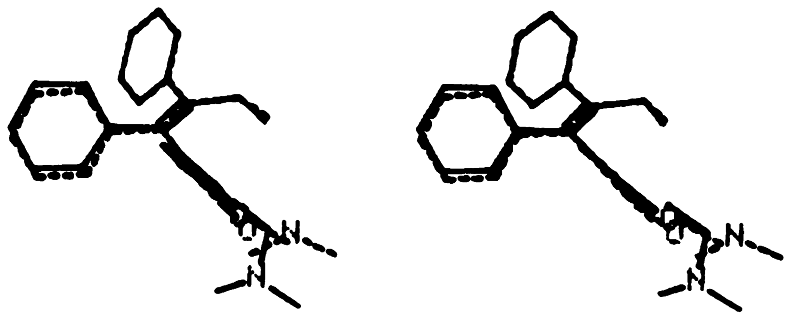

FIGURE 11. Comparison of the two crystallographically observed conformers of *cis* (E) tamoxifen illustrating the similarity in the substituted ethylene group and variations in the alkylamine-ethoxy side chain orientation.

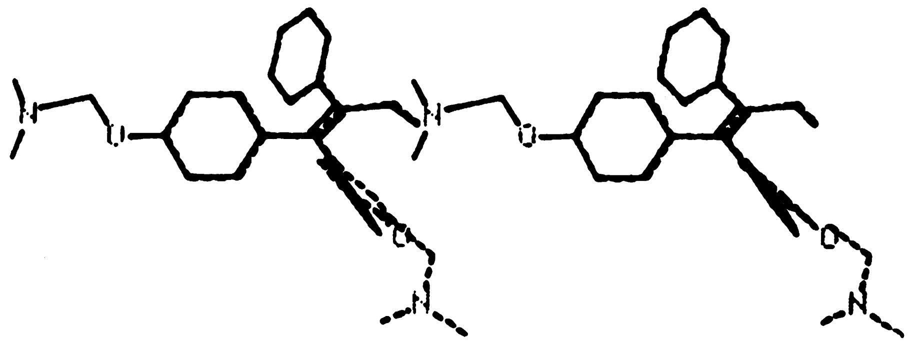

FIGURE 12. Comparison illustrating the similarity of the observed conformations of E and Z tamoxifen with β rings superimposed.

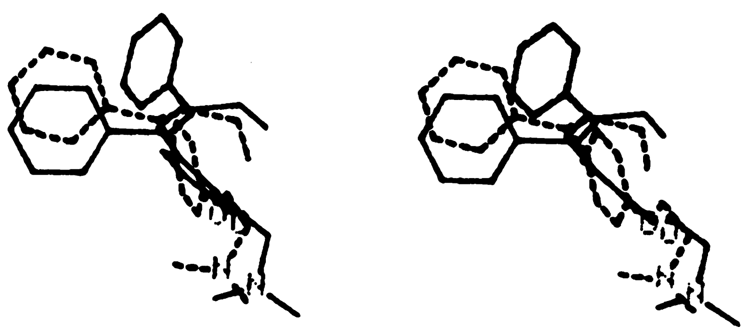

FIGURE 13. Comparison of observed and calculated conformations of *cis* tamoxifen, with β rings superimposed.

would bind to the receptors in one of the two ways illustrated in Figure 14. In either orientation, the absence of oxygen substitution comparable to O(17) on estradiol may account for the inactivity/antagonism of the compound. In addition to this, the amino-substituted ring may present steric interference to a molecular event (i.e. conformational change in the receptor or genomic interaction) that is essential to hormone action. It may be that when bound to the receptor in one of these orientations *trans* tamoxifen conveys antiestrogenic activity, while in the other form it is merely an impeded weak agonist.

Summary

The X-ray crystallographic structural determinations of synthetic estrogens and antiestrogens described here provide reliable information on the global minimum energy conformation of these molecules or a local minimum energy conformation that is within one or two kilocalories per mole of the global minimum. The similarity in multiple observations of DES, of INDA and of INDB, despite extensive variation in intermolecular interactions indicates that these molecules have well defined minimum energy conformations that exhibit minimal distortion as a result of crystal packing interactions. This conclusion is consistent with those previously drawn on the basis of examination of the 17 side chain of 85 pregnane structures (*5*) and an accurate low temperature neutron diffraction study of 20-methyl-5-pregnene-3β,20-diol (*23*). In favorable cases, state-of-the-art molecular mechanics calculations provide quantitative agreement with X-ray results and information on the relative energy of other local minimum energy conformations not observed crystallographically. While the

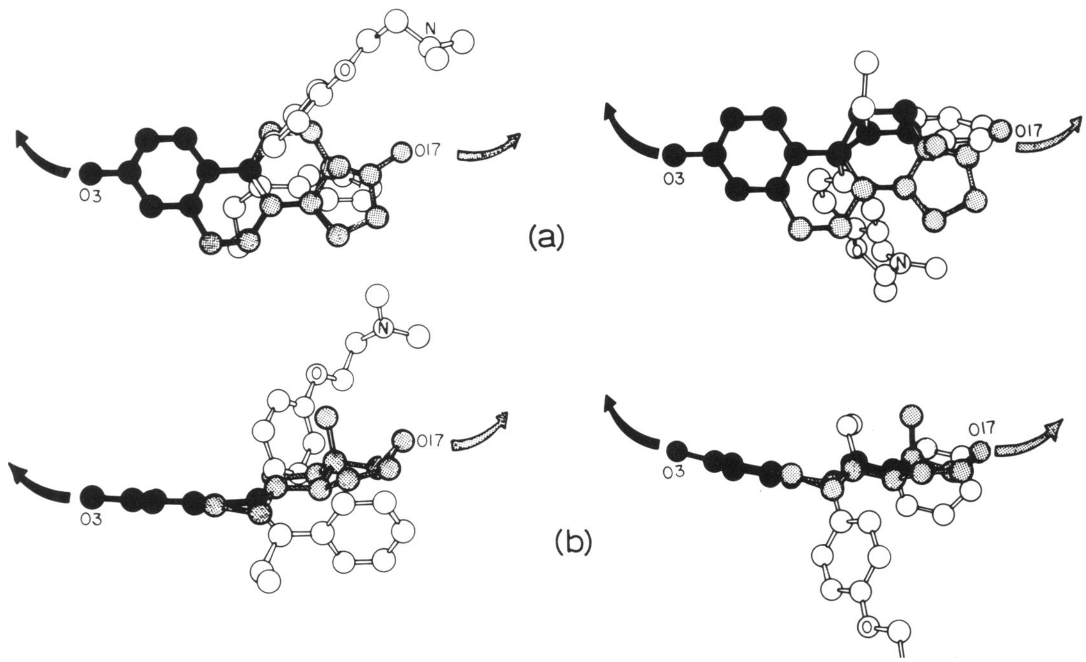

FIGURE 14. Comparison of the crystallographically observed conformation and hydrogen bonding of estradiol with that of *trans* tamoxifen. For purposes of the comparison, the A ring of estradiol is superimposed on the α′ phenyl ring of tamoxifen that is hydroxylated in animal metabolism. The two possible superpositions of these rings are illustrated in (*a*) and (*b*). Common structural features are dark, estradiol structure is shaded, and *trans* tamoxifen is light.

MM2p program provides reliable information on bond lengths, angles, and some torsional angles, it fails to minimize correctly the torsional relationship for C(sp^3)-C(sp^2) formal single bonds.

Because the conformation of DES observed in solvated crystals has an overall conformation and dipole moment more similar to estradiol it is the form more likely to bind to the receptor and produce hormone activity. Either phenol ring of DES can successfully mimic the estradiol A-ring in binding to the receptor. Indenestrol A and indenestrol B have nearly identical fully extended planar conformations. Either the α or γ rings of these compounds may mimic the A ring of estradiol and compete for the estrogen receptor. Although there are eight distinct ways in which molecules of a racemic mixture of INDA or INDB can bind to the receptor, not all of them may be able to elicit a hormonal response. This may account for the reduced biological activity of the compounds despite their successful competition for receptor binding. The bent conformation of indanestrol together with the relative instability of an extended conformer are consistent with the low receptor affinity and lack of activity of this compound.

The minimum energy conformations of ZPD and EPD are bent in a fashion similar to that of INDC. These molecules have good binding affinity even with the bent shape, suggesting that the receptor does not require a flat molecule. Therefore these conformations would appear to be compatible with receptor binding, but only the Z isomer has an energetically allowed extended conformation that accounts for its observed biological activity relative to DES. The phenolic ring of the monohydroxylated metabolites of the potent antiestrogen Z-tamoxifen almost certainly mimics the steroid A-ring in binding to the estrogen receptor. When this ring is superimposed upon the A-ring of estradiol, it is clear that the antiestrogenic character of Z-tamoxifen stems from the absence of a hydroxyl group located in a position analogous to that of the 17β-OH in estradiol. The additional phenyl ring may interfere with an activity controlling macromolecular interaction that follows receptor binding.

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The MM2p program is derived from the programs MM2 and MMP1 that have been developed by Norman Allinger (*13,14*) and are available from the Quantum Chemical Program Exchange. The MM2p program incorporates the Variable Electronegativity Self-consistent Field portion of the MMP1 program into the MM2 program.

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